# SYNTHESIS AND BIOCOMPATIBILITY EVALUATION OF GELATIN BASED HYDROGELS

*Thesis submitted for the award of the degree of*

**BACHELOR OF TECHNOLOGY**

*by*

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# CERTIFICATE

DATE:14/05/2012

This is to certify that the thesis entitled "**SYNTHESIS AND BIOCOMPATIBILITY EVALUATION OF GELATIN BASED HYDROGELS**" submitted by **Mr. PRADEEP BHAGAT (108BT009)**, in partial fulfillment of the requirements for the degree of B.Tech in Biotechnology, embodies the bonafide work done by him in the final year of his degree under my supervision. The thesis or any part of it has not been submitted earlier anywhere for the award of any degree or diploma.

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# ABSTRACT

The research study describes the development of gelatin based hydrogels. Four different hydrogels were prepared by varying poly-ethylene glycol (PEG) and gelatin ratios. The hydrogel samples were characterized by FT-IR technique. The hydrogels were evaluated by swelling studies, mucoadhesive test, antimicrobial growth and haemocompatibility test. Metronidazole drug was incorporated within hydrogels and their *in-vitro* drug release behavior was studied.

Swelling studies of hydrogels were carried out in PBS solution of pH = 7.2. It was observed that with increase in volumetric ratio of gelatin to PEG, there is increase in swelling behavior. Mucoadhesive study showed that all these hydrogel samples were highly mucoadhesive. Mucoadhesivity was found to be less with lower gelatin content. Antimicrobial studies using *B. Subtilis* showed that drug loaded hydrogel samples exhibited better zone of inhibition than the blank hydrogels. The haemocompatibility test was carried out using goat's blood. The percentage of haemolysis was found to be less than 5 percent which indicates that all these hydrogels are highly haemocompatible with blood.

Drug release studies were carried out in the hydrogels by incorporating Metronidazole drug. It was observed that with increase in gelatin:PEG volumetric ration, there is increase in amount of drug release.

Keywords: hydrogels, swelling studies, mucoadhesivity, haemocompatibility, drug delivery

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# ABBREVIATIONS AND SYMBOLS

| SN | ABBREVIATIONS OR SYMBOLS | FULL NAME |
|---|---|---|
| 1. | PEG | Poly(ethylene glycol) |
| 2. | A | Hydrogel sample with 7:13 ratio of PEG: Gelatin |
| 3. | B | Hydrogel 8:12 |
| 4. | C | Hydrogel 10:10 |
| 5. | D | Hydrogel 15:5 |
| 6 | DA | Drug loaded hydrogel sample with 7:13 ratio of PEG: Gelatin |
| 7 | DB | Drug loaded hydrogel 8:12 |
| 8 | DC | Drug loaded hydrogel 10:10 |
| 9 | DD | Drug loaded hydrogel 15:5 |
| 10. | S & S(sample code) | Swelling ratio & swelling ratio of particular sample |
| 11 | $D_T$ | Disintegration time |
| 12 | O.D | Optical density |
| 13 | FT-IR | Fourier transform infrared radiation |
| 14. | $O.D_{+VE}$ | Optical density of +ve control |
| 15. | $O.D_{-VE}$ | Optical density of -ve control |
| 16. | FT-IR | Fourier transform infrared spectroscopy |
| 17. | $SW_0$ | Dry(initial) weight of hydrogel sample |
| 18. | $W_T$ | Swollen weight of hydrogel sample at time T |
| 19. | S | Swelling ratio |

# Chapter 1

# INTRODUCTION AND OBJECTIVE

## ✓ INTRODUCTION

Hydrogels are 3-D cross-linked networks of water soluble polymers. The hydrogels can be defined as a cross-linked polymeric network which has the capacity to hold water within its porous structure. The water holding capacity arises mainly due to the presence of hydrophilic groups, viz. amino, carboxyl and hydroxyl groups, in the polymer chains. Polymers can be made from virtually any water soluble polymer encompassing a wide range of chemical compositions and bulk physical properties. The hydrogels are widely used in clinical practice and in experimental medicines in a very wide range e.g. diagnostics, cellular immobilization or separation of biomolecules or cells and barrier materials to regulate biological adhesions.

Hydrogel is very significant for the drug delivery because of its unique physical properties. Tuning of highly porous structure can be done by controlling the density of cross links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Loading of drugs in the gel matrix is permitted by their porosity and subsequent drug release at a rate dependent on diffusion coefficient of the small molecule or macromolecule through the gel network. Hydrogels are highly bio-compatible, the high water content and physiochemical similarities to the native extracellular matrix promotes the bio-compatibility of the hydrogels [1]. With many advantageous properties, hydrogels have few limitations such as well:

a. Low tensile strength
b. High water content
c. Large pore size

These are some of the problems associated with hydrogels, but the supporting properties of hydrogel for its application are utilized in such a way that these problems effects become negligible. Hydrogels are highly absorbent synthetic polymers. Hydrogels which exist naturally inside the body are mucus, vitreous humor of eye cartilage, tendons and blood clots etc.

## 1.1 CLASSIFICATION OF HYDROGELS

On the basis of their occurrence and synthesis, hydrogels are broadly classified into three types. They are:

a. Natural hydrogels
b. Synthetic hydrogels
c. Semisynthetic hydrogels

### 1.1.1 Natural hydrogels

These are hydrogels that exist naturally in the environment and need not to be synthesized. Examples of naturally occurring hydrogels are mucus, vitreous humor of eye cartilage, tendons and blood clots.

### 1.1.2 Synthetic hydrogels

These hydrogels are synthesized artificially and has similar properties to that of the natural hydrogels. Synthetic hydrogels have been considered as potential candidates for mimicking life.

### 1.1.3 Semisynthetic hydrogels

Those hydrogels prepared by the partial chemical synthesis are known as semi synthetic hydrogels. Need of semi synthetic hydrogels arises when precursor molecule is too structurally complex or costly to be produced by total synthesis. It is also possible that the semisynthetic out-performs the original biomolecule itself with respect to potency, stability or safety. In such cases semisynthetic hydrogels can itself perform the task of natural hydrogels.

On basis of nature, hydrogels are classified into the following pH sensitive, temperature sensitive, enzyme sensitive and electrical sensitive.

## 1.2 APPLICATION OF HYDROGELS

Hydrogels have extensive application in regenerative medicine, mainly because of its structural similarity to that extracellular matrix. As a result they can be effectively applied in the body. Some of the other properties which support their wide application are its biocompatibility, mechanical strength, swelling properties, mucoadhesivity, haemocompatibility etc. Some of its applications are:

a. They are used as scaffolds in tissue engineering.
b. Environmentally sensitive hydrogels which are known as smart gels or intelligent gels. These hydrogels have the ability to sense pH, temperature or the concentration of metabolite and release their load as a result of such a change.
c. Those which are responsive to specific molecules such as glucose or antigens can be used as biosensors [2].
d. They can also be used as contact lenses e.g. silicone hydrogels, polyacrylamide.
e. EEG uses hydrogels composed of cross linked polymers.

## 1.3 GELATIN

Gelatin is a gelling agent and has the following properties like colorless, flavorless, brittle (when dry). Gelatin is a hydrolysed form of collagen. It is a mixture of proteins and peptides. It is used as a gelling agent in food, pharmaceuticals, photography and cosmetic manufacturing. Gelatin is an irreversibly hydrolyzed form of collagen. Household gelatin comes in form of sheets, granules or powder.

## 1.4 PEG (POLYETHYLENE GLYCOL)

PEG is a polymer which is formed by polymerization of ethylene oxide (Fig. 1). It is a non-toxic water soluble polymer. PEG has a wide range of industrial and biomedical applications. Its biological activity is contributed by coupling of biological molecule to PEG. Proteins that are used with the PEG have a high clearance rate and do not get denatured. On the basis of number of arms PEG is divided into three types. They are

a. Branched PEG (3-10 number of arms)
b. Star PEG (10-100 number of arms)
c. Comb PEG (above 100 arms)

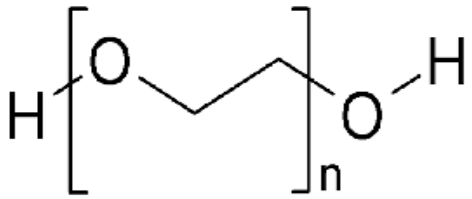



**Fig. 1: Chemical structure of polyethylene glycol**

## 1.5 OBJECTIVE

The main objective of the current research project is

a. Preparation of a gelatin-based hydrogel of different composition.
b. To evaluate hydrogels biocompatibility through swelling studies, mucoadhesivity test, antimicrobial growth and haemocompatibility test.
c. To study the rate of drug release using Metronidazole as a drug.

# Chapter 2

# LITERATURE REVIEW

## 2 LITERATURE REVIEW

Hydrogels are polymeric networks which hold water and can be used in various application like drug delivery. The hydrogel property depends on the swelling and diffusion capacity of the film. The hydrogels have a good capability of swelling or retaining water inside itself. The ability of hydrogels to absorb water is due to the presence of hydrophilic groups such as –OH, -COOH, -$SO_3H$, -CONH, -$CONH_2$. The various properties of hydrogel which have direct relation with the swelling behavior are permeability, mechanical properties, surface properties, and biocompatibility. Hydrogels matches to great extent in their physical properties to that of living tissue, and this similarity is due to the high water content, soft and rubbery consistency and low interfacial tension with water or biological fluids. Various factors which can be used to control the release rate and release mechanisms from the hydrogels are polymer composition, crosslinking density, crystallinity, water content etc. [3].

Gelatin is a mixture of proteins and peptides which can be produced by hydrolysis of collagen obtained from the skin, connective tissues , organs and some intestines of animals. Gelatin gels exist over only a small temperature range, the lower limit the freezing point and the upper limit being the melting point of the gel. The production of gelatin consists of 3 main steps

1. Pretreatments to remove impurities and make the raw materials ready for the main extraction step.
2. The main extraction step is done to hydrolyze collagen into gelatin.
3. The refining and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding, and sifting to remove the water from the gelatin solution.

PEG is a non-toxic water soluble polymer. They are found in the liquid state or in a very low melting solid. Their melting point is very less. PEG are excellent candidates as biomaterials. PEG may transfer its properties to another molecule when it is covalently bound to that molecule. Coupling of biological molecule to PEG contributes to its biological activity [2].

When a hydrogel matrix is exposed to the aqueous medium, three regions will generally be

distinguishable within the hydrogel matrix:

a. Mostly hydrogel

b. Significant proportions of water and hydrogel

c. Mostly water

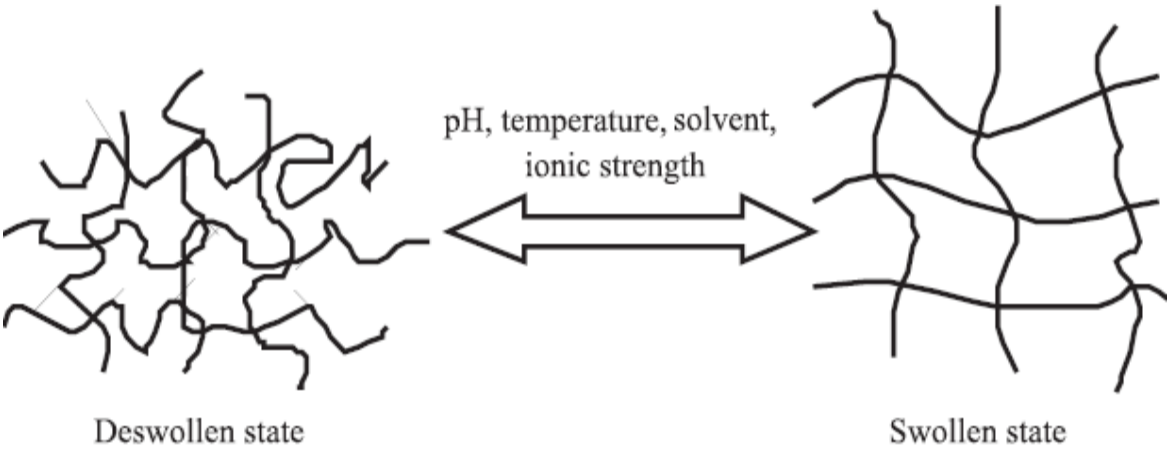



**Fig. 2: Schematic representation of hydrogels swelling.**

A swelling agent is a three dimensional network of hydrophilic chains that are chemically or physically cross linked. Depending on their structure swelling agents can absorb aqueous or organic solutions. If former is the case swelling agent is known as hydrogel. The driving force for absorption or swelling process is generally a balance of three forces

a. Osmotic

b. Electrostatic

c. Entropy-favoured dissolution of polymer in water.

Highly cross-linked hydrogel networks display faster swelling compared to their lightly cross-linked counter parts [5]. Three major elements control the swelling process of a hydrogels are

i) Crosslink content

ii) Ionic content

iii) hydrophilic content

Mucoadhesion is the property of a material by which it adheres to the surface of mucus. Mucosal surfaces such as in the gut or nose are covered by a layer

of mucus. Mucoadhesive agents are usually polymers containing hydrogen bonding group. The reason behind mucoadhesion have not yet been fully understood, but a basic accepted theory is that close contact must first be established between the mucoadhesive agent and the mucus, followed by interpenetration of the mucoadhesive polymer and the mucin and finishing with the formation of entanglements and chemical bonds between the macromolecules.

Antimicrobial activity shows the inhibition property of the materials to the micro-organism. The efficacy of the gels as a carrier for antimicrobial agents can also be studied against *B. subtilis*, gram positive bacteria. The antimicrobial activity is basically determined by agar diffusion assay. Larger the zone of inhibition of the substance greater is its anti-microbial activity.

It is very important to consider when devices are designed for applications that come in contact with blood. Events that determine haemocompatibility often occur at the molecular level. Haemocompatibility can influence inflammatory processes. The % haemolysis is calculated and it basically exhibits the amount of red blood cells which are dead.

Drug delivery is one of the major applications of hydrogels. There are basically two ways that a drug can be released

a. Degradation of implant
b. Diffusion through implant

The hydrogels are loaded with known concentration of drug molecule. When there is diffusion, U.V spectrophometry is used to quantify the release of drug molecule. Spectrophotometry is a method of characterizing solution concentration by measuring the amount of light that is transmitted through a sample [4]. A standard curve equation for the particular drug is used to calculate the amount of drug released.

# Chapter 3

# MATERIALS AND METHODS

## 3.0 MATERIALS

The reagent grade chemicals used during the synthesis process are

a. Gelatin →HIMEDIA, MUMBAI, INDIA
b. PEG and Tri-Sodium citrate →LOBA CHEMIE PVT. LTD MUMBAI
c. Ethanol . →HONYON INTERNATIONAL INC, HONGYANG CHEMICAL CORPN. CHINA
d. Glutaraldehyde and HCl 35% pure → MERCK SPECIALITES PVT. LTD MUMBAI INDIA
e. Goat intestine and blood → LOCAL BUTCHER SHOP
f. Metronidazole → VEEVEES TRADE IMPEX PVT. LTD INDIA
g. Double distilled water was used throughout the experiment.

## 3.1 PREPARATION OF HYDROGELS

The hydrogels are prepared using the following chemicals: Poly ethylene glycol, Gelatin, Glutaraldehyde, Ethyl alcohol, 35% pure HCl**.** Cross linker was prepared by mixing the chemicals in a specific volumetric ratio i,e. (Glutaraldehyde **:** Ethanol **:** HCl = 1 **:** 1 **:** 0.2 ). 10% w/v of PEG and gelatin is prepared by dissolving 10 g of PEG in 100 ml of water in a beaker and 1 g of Gelatin in 100 ml of water in separate beaker. Four different compositions of gelatin**:** PEG were taken and coded as shown in (Table 1). It is then stirred in a magnetic stirrer until it becomes a homogeneous solution. Then cross linker with specific ratio is put into the solution and kept for 3 minutes. The solution is then collected and poured into petridish for 24 h to form hydrogels.

**Table 1: Hydrogels with different compositons**

| SN | SAMPLECODE | VOL. OF 10%PEG(W/V)(ml) | VOL. OF 10%GELATIN(W/V)(ml) |
|---|---|---|---|
| **1.** | **A** | **7** | **13** |
| **2.** | **B** | **8** | **12** |
| **3.** | **C** | **10** | **10** |
| **4.** | **D** | **15** | **5** |

## 3.2 FT-IR Analysis

**Sample preparation**

Hydrogel samples A and D were made into powdered form with the help of liquid nitrogen. The hydrogels were thoroughly powdered and dried under vacuum at 60ºC for 24 hours before mixing with anhydrous KBr. It is then made into discs in a KBr press. The samples are now ready to be used for the FT-IR analysis.

**Method**

FT-IR spectra of hydrogel samples A and D in KBr disc form were recorded on Perkin-Elmer 2000 FTIR spectrometer between 400 to 4000 $cm^{-1}$ with a resolution 4 $cm^{-1}$ at 100 scans.


**(a)** **(b)**

**Fig. 3: (a) FT-IR Spectrometer (b) KBr press for pelletizing**

## 3.3 SWELLING STUDIES

Hydrogel is a three dimensional network which has the property to absorb water or any other liquid solutions. So to compare the swelling properties of hydrogels having different compositions swelling measurement was done at pH 7.2 (as most of the body fluids have their pH quite equivalent to 7.2).

**Sample preparation** Square shaped small pieces of the four hydrogel samples (A, B, C and D) weighing 1 g each were cut with the help of blade. 10 ml of PBS solution was poured into each of 4 petridishes. All the four hydrogel samples weighing 1 g were dipped

into the petridishes and its weight was measured for every 15 minutes of interval time. The experiment was continued until the hydrogels were saturated and there was no increase in their weight.

The swelling ratio was calculated using the formula:

$$S = (W_{t.} - W_0)/W_0$$

where,

S = Swelling Ratio

$W_{t.}$ = Weight of hydrogel sample at time, t

$W_0$ = Initial weight of the hydrogel sample at time, t=0

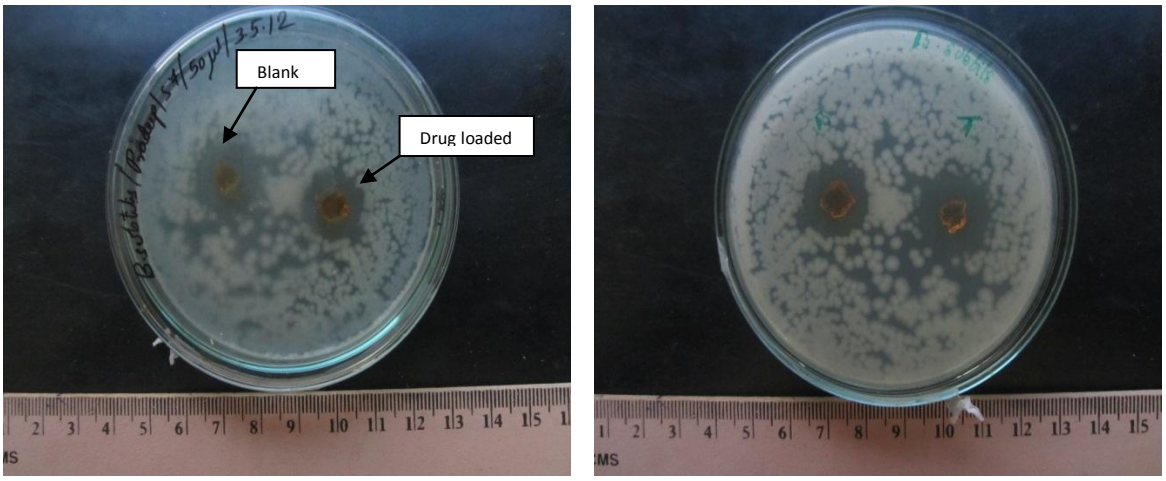
## 3.4 MUCOADHESIVITY TEST

Mucoadhesivity is the property which shows the degree of adhesion of a substance to the mucus layer. Small square pieces (1cm x 1cm) of hydrogel samples A, B, C and D were cut with the help of a blade. Intestine of a freshly cut goat was bought from the butcher shop and immediately transferred to cold saline solution. The intestine was cut open and adhered to glass slides with the help of glue such that the intestinal mucosa exposed outwards. The hydrogel samples were mounted on the top of the mucosa surface. A weight of 5 g was applied over the hydrogel samples for 5 min to ensure its adherence to mucosa surface. The glass slides were were transferred to USP Disintegration baskets (as shown in Fig. 4). Phosphate buffer solution (PBS) was used as disintegration medium. The disintegration was checked in intervals of 1 h for 24 h.

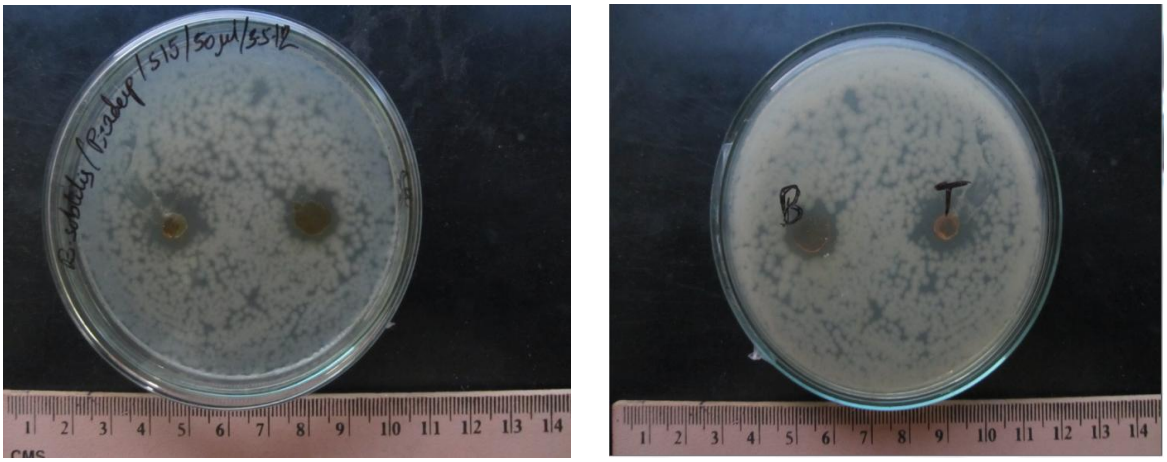

**Fig 4 : Tablet disintegration apparatus.**

## 3.5 ANTIMICROBIAL TEST

Gram positive bacteria *Bacillus Subtilis* was used to analyze the antimicrobial activity of the blank hydrogel sample (without drug) and drug loaded hydrogel (metronidazole) respectively. Solid nutrient agar of 1 ml was used as the media. Four petriplates and nutrient agar medium were autoclaved and kept in the laminar hood flow along with the samples for 15 minutes. The inoculation of micro-organism was done by spread plate method. 0.5 g of each sample (blank and drug loaded) was put into the petriplates. The petriplates were then incubated at 37 °C for 24 h. The zone of inhibition (as shown in Fig. 5) was measured using a scale after incubation.




**a.**



**b.**

**Fig 5: a. Antimicrobial growth on hydrogel sample A**
**b. Antimicrobial growth on hydrogel sample D**

## 3.6 HAEMOCOMPATIBILITY TEST

The hydrogel samples were first washed with water continuously for 2-3 times and was mixed with 0.5 ml of citrated goat blood diluted with normal saline (prepared in 4:5 ratio), followed by the addition of normal saline (9.5 ml). The positive control (+ve) was prepared by adding 0.5 ml of 0.1 N hydrochloric acid to 0.5 ml of diluted blood. The negative control (-ve) was prepared by adding 0.5 ml of saline to 0.5 ml of diluted blood. The final volume was made up to 10 ml using saline. The +ve control and the –ve control were incubated at 37 $^{o}$C for 1 h as shown in Fig. 6. After incubation, the samples were subsequently centrifuged at 3000 rpm for 10 min. The supernatant was analyzed at 545

nm using a UV-visible spectrophotometer (UV 3200 double beam, Lab India). The percentage of haemolysis was calculated from the following equation:

$$\% \text{ HEMOLYSIS} = ( O.D_{TEST} - O.D_{-VE} / 0.D_{+VE} - O.D_{-VE}) * 100$$

where,

$O.D_{TEST}$ = O.D of sample

$O.D_{-VE}$ = O.D of –ve control

$O.D_{+VE}$ = O.D of +ve control

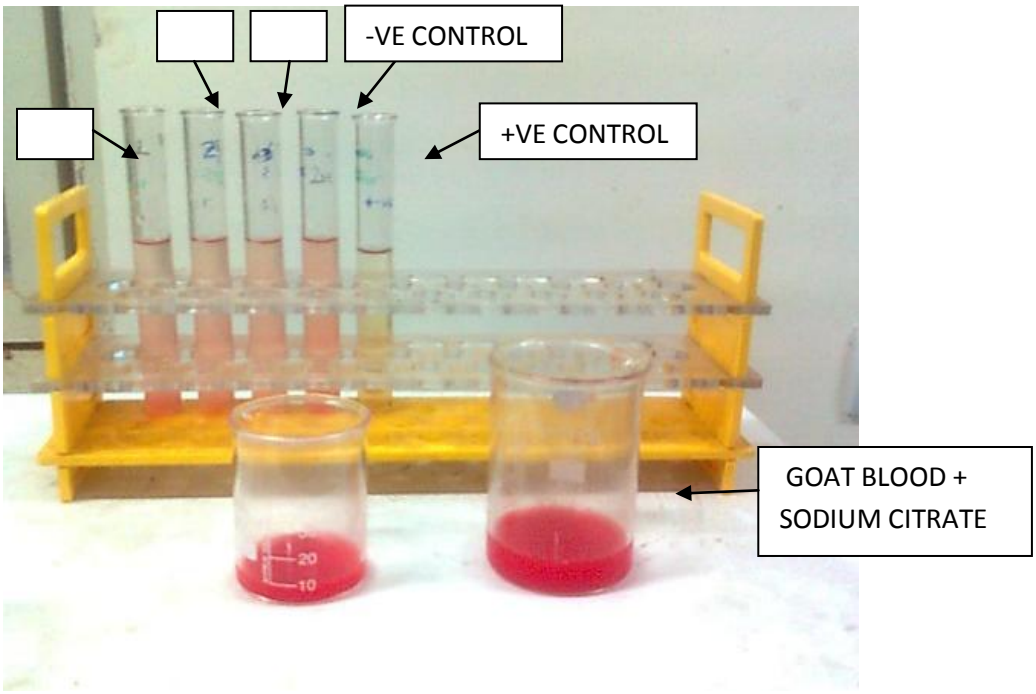



**Fig 6: Test tubes having test samples , +ve control and –ve control.**

## 3.7 IN-VITRO DRUG RELEASE

Drug release study was carried out in the dissolution rate test apparatus. Metronidazole drug loaded hydrogel samples of similar compositions of normal hydrogels (A, B, C and D) were synthesized and coded as DA, DB, DC, and DD. The drug loaded hydrogels were cut into 20x20mm dimensions and taken in 900 ml beaker filled with distilled water as shown in Fig. 7). The temperature is maintained at 37°C and stirred at the rate of 100 rpm. For every interval of 15 min, 5 ml solution from the dissolution flask was removed and replaced by 5ml of distilled water. This process is carried out up to 1 h. After that 5 ml of sample was removed from the dissolution flask for every 30 min up to 5 h. Every time when 5 ml solution is removed its O.D was measured using U.V spectrophotometer. This procedure was repeated for all the 4 compositions. The amount of drug released was calculated from the standard curve equation of Metronidazole [6].

$$\mathbf{Y = 0.058\ X}$$

where, Y = O.D of the sample solution

X = Amount of drug released


**Fig 7: Dissolution rate test apparatus**

# Chapter 4

# RESULTS AND DISCUSSION

# 4. GELATION OF HYDROGEL SAMPLE

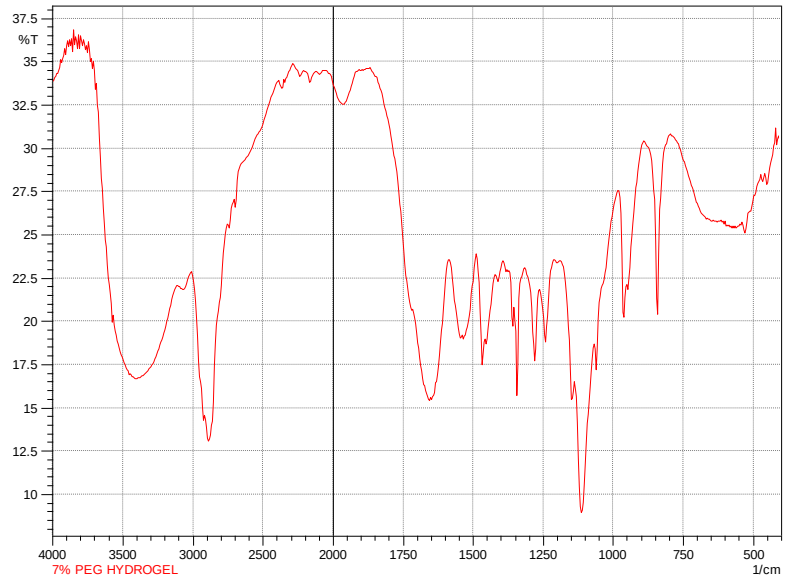
The various compositions of the hydrogels have been tabulated in table. These hydrogel samples vary in their volumetric ratio of Gelatin to PEG. It was found that all the samples A, B, C and D resulted in formation of hydrogels.

**Table 2: Gelation property of all 4 hydrogels**

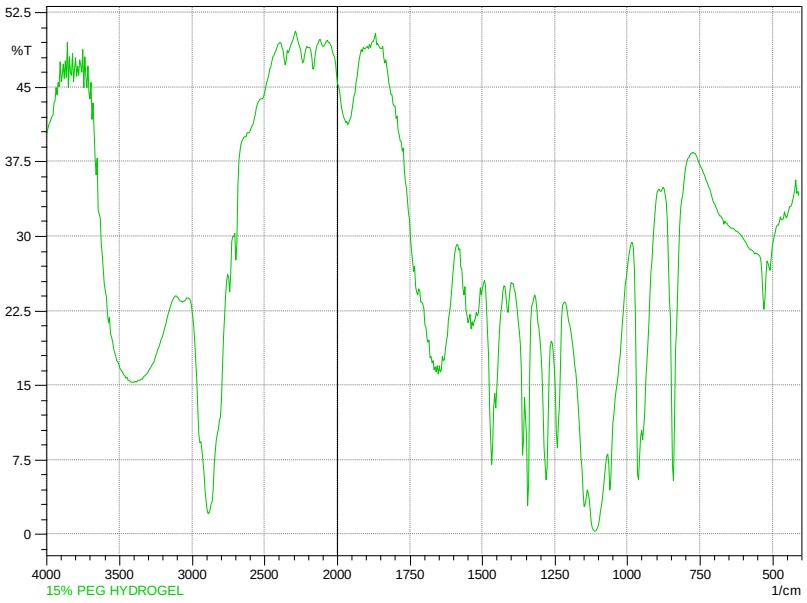
| S .N | SAMPLE CODE | VOL. OF 10%PEG (W/V) (ml) | VOL. OF 10%GELATIN (W/V) (ml) | GELATION PROPERTY |
|---|---|---|---|---|
| 1. | A | 7 | 13 | Hydrogel formed |
| 2. | B | 8 | 12 | Hydrogel formed |
| 3. | C | 10 | 10 | Hydrogel formed |
| 4. | D | 15 | 5 | Hydrogel formed |

## 4.1 FT-IR ANALYSIS

FT-IR result shows that there is formation of new bonds which leads to create absorption at different wave numbers. It can be seen in the graph of FT-IR results that the peaks for both the sample A and D are at same wave numbers (Fig. 8). The difference is in their intensity of transmittance. Sample D has higher intensity as compared to sample A. The peaks (as shown in the graph) at specific wave numbers refer to bonds or linkage of functional groups and occurrence of vibration which leads to absorption. Various linkage and other factors related to peaks are tabulated in the Table 3.




(a)




**(b)**

**Fig. 8 : (a) FTIR result for sample A and (b) FTIR result for sample D**

**Table 3: Infra-red Absorption frequencies occurrence in sample A and D.**

| S N | WAVE NUMBER AT PEAKS ($CM^{-1}$) | INTENSITY | ASSIGNMENTS |
|---|---|---|---|
| 1. | 3452 | Weak | NH ($1^{O}$-Amines), 2 bands |
| 2. | 2855 | Strong | $CH_3$, $CH_2$, & CH , 2 OR 3 bands |
| 3. | 1937 | Strong | C=C(asymmetric stretch) |
| 4. | 1631 | Variable | C=C(Symmetry reduces intensity) |
| 5. | 1514 | Medium | NH ($2^{O}$ – Amide) |
| 6. | 1441 | Strong | A – $CH_2$ bending |
| 7. | 1339 | Medium | O-H bending in plane |
| 8. | 1266 | Med. – Str. | O-C sometimes 2 peaks |
| 9. | 1223 | Med. – Str. | O-C sometimes 2 peaks |
| 10. | 1092 | Medium | C-N |
| 11. | 931 | Strong | =C-H & $=CH_2$ |
| 12. | 844 | Medium | Out of plane bending |

## 4.2 SWELLING STUDIES

From swelling studies, it was found that the hydrogels has a great capacity to store water or any other similar solvent, but it has an ending point at which the hydrogel gets saturated and there is no increase in the weight furthermore. In this study, it was observed that the swelling ratio was more for the same time period for the sample whose concentration of gelatin was high (sample A) i.e S(A) > S(B) > S(C) > S(D) respectively.

**Table 4 : Data of swelling study at pH 7.2**

| S N | TIME(MIN.) | S(A) | S(B) | S(C) | S(D) |
|---|---|---|---|---|---|
| **1.** | 0 | 0 | 0 | 0 | 0 |
| **2.** | 15 | 0.13 | 0.1 | 0.07 | 0.04 |
| **3.** | 30 | 0.25 | 0.23 | 0.16 | 0.1 |
| **4.** | 45 | 0.37 | 0.34 | 0.23 | 0.16 |
| **5.** | 60 | 0.46 | 0.43 | 0.32 | 0.21 |
| **6.** | 90 | 0.55 | 0.54 | 0.4 | 0.26 |
| **7.** | 120 | 0.65 | 0.63 | 0.49 | 0.32 |
| **8.** | 150 | 0.77 | 0.74 | 0.57 | 0.39 |
| **9.** | 180 | 0.9 | 0.82 | 0.66 | 0.46 |
| **10.** | 210 | 1.05 | 0.93 | 0.75 | 0.51 |
| **11.** | 240 | 1.05 | 0.93 | 0.75 | 0.51 |

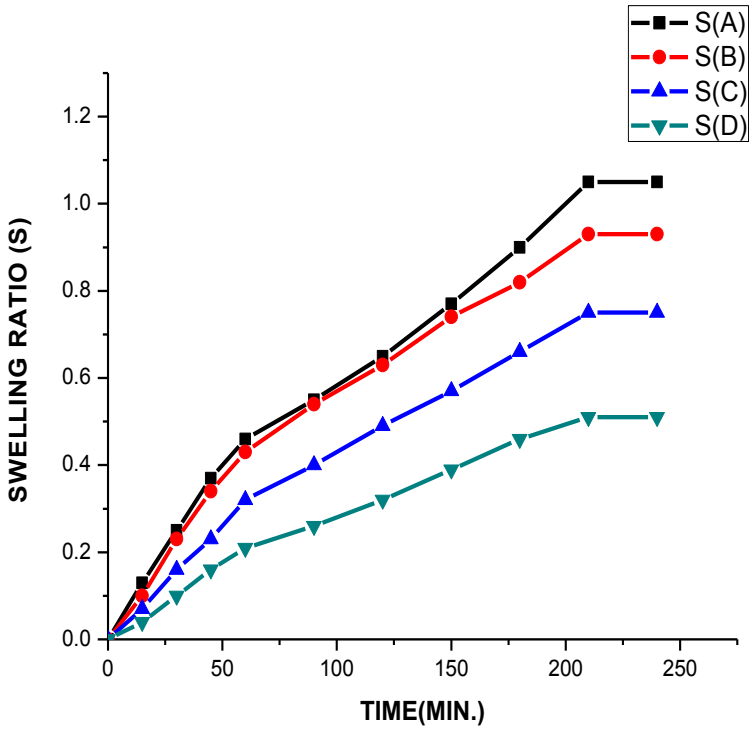



**Fig. 9: Swelling studies of various hydrogels at pH 7.2**

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## 4.3 MUCOADHESIVITY TEST

After completion of 24 h the result was found that three of hydrogel samples A, B, and C still adhered to the mucus layer and sample D was found disintegrated from the mucus layer. Sample A, B and C shows highly mucoadhesive where as sample D is not. The reason could be the concentration of gelatin which is quite high in A, B and C which provides the samples with mucoadhesive properties to make it highly mucoadhesive. The disintegration time for all the samples are tabulated in the Table 5. The order of distintegration is as follows:

$$D_T(D) < D_T(C) \leq D_T(B) \leq D_T(A)$$

**Table 5: Results of disintegration time of the Hydrogels**

| SN | SAMPLES | $D_T$ (h) |
|---|---|---|
| 1. | A | 24 |
| 2. | B | 24 |
| 3. | C | 24 |
| 4. | D | 20 |

## 4.4 ANTIMICROBIAL EVALUATION TEST

The zone of inhibition for all 4 samples was measured at three different places and the averages of 3 values were tabulated in Table 6. The study showed that the PEG hydrogels had a mild activity against *Bacilus subtilis*. The drug loaded hydrogels were found more active compared to blank hydrogels in their ability to restrict the growth of the microorganisms in the surrounding area known as zone of inhibition of the formulation and did not allow the growth of microorganisms even after 24 h. This is because drug loaded hydrogels has the effect of drug too which increases the antimicrobial property of the hydrogels where as blank hydrogel has solely its own anti microbial property. Diameter of zone is correlated inversely with the minimum inhibitory concentration of antibiotic for that bacteria, so large the zone is smaller will be minimum inhibitory concentration and vice versa. From the result of antimicrobial evaluation, the hydrogels were found to be antimicrobial and restricts the growth of microbes in a certain zone (as shown by the radius of each samples zone of inhibition). Comparing the results of all these samples, it was found that zone of inhibition increases with the increase in volumetric concentration of gelatin**:** PEG ratio.

**Table 6: Result of Antimicrobial evaluation**

| SN | SAMPLECODE | ZONE OF INHIBITION(Averagevalues) | |
|---|---|---|---|
| | | BLANK | TEST |
| 1. | A | 1.1±0.1 | 1.4±0.2 |
| 2. | B | 1.1±0.1 | 1.4±0.2 |
| 3. | C | 1.0±0.1 | 1.2±0.2 |
| 4. | D | 0.9±0.1 | 1.2±0.2 |

## 4.5 HAEMOCOMPATIBILITY TEST

Absence of haemolysis is an indication of good haemocompatibility. The % haemolysis indicates the extent of lysis of red blood cells when kept in contact with blood. The % haemolysis of all the 4 samples were calculated by the % haemolysis formula and tabulated in Table 7. It was observed that all the 4 samples shown % haemolysis value less than 5 (standard ASTM value) this suggests high haemocompatibility on the preliminary study.

**Table 7: Result of % of haemolysis for various hydrogels**

| SN | SAMPLE CODE | $O.D_{test}$ | $O.D_{-VE}$ | $O.D_{+VE}$ | % HEMOLYSIS |
|---|---|---|---|---|---|
| 1. | A | 0.010 | 0.009 | 0.137 | 0.78 |
| 2. | B | 0.012 | 0.009 | 0.137 | 2.34 |
| 3. | C | 0.012 | 0.009 | 0.137 | 2.34 |
| 4. | D | 0.012 | 0.009 | 0.137 | 2.34 |

## 4.6 DRUG RELEASE

The drug release studies were continued for 5 h in the dissolution rate test apparatus. It was found that the amount of drug delivery increases with increase in the % of gelatin. In this case four samples were tested for their drug release activity varying in the ratio of gelatin **:** PEG as shown in Fig. 10. The sample A has the ratio 13**:7** which has the greater drug release in comparison to the other samples. The other 3 samples also have their drug release activity decreasing in order of their decreasing Gelatin: PEG ratio. It was also found that greater the difference in the concentration of the gelatin greater is the difference in their drug release activity. As the drug release graph for the samples having little difference in their concentration of gelatin i.e. conc. is 7% , 8% and 10% respectively are quite closer to each other while the graph for the sample with concentration 15% has a quite much difference among them as compared to other so has its graph quite far as per comparison with other samples.

From the above results it can be inferred that drug interferes with the forming of film which leads to shrinkage of the hydrogel film removing the excess water outside. The concentration of gelatin also plays a vital role in forming the films. As the gelatin concentration decreases the hydrogel with drugs shrinks.

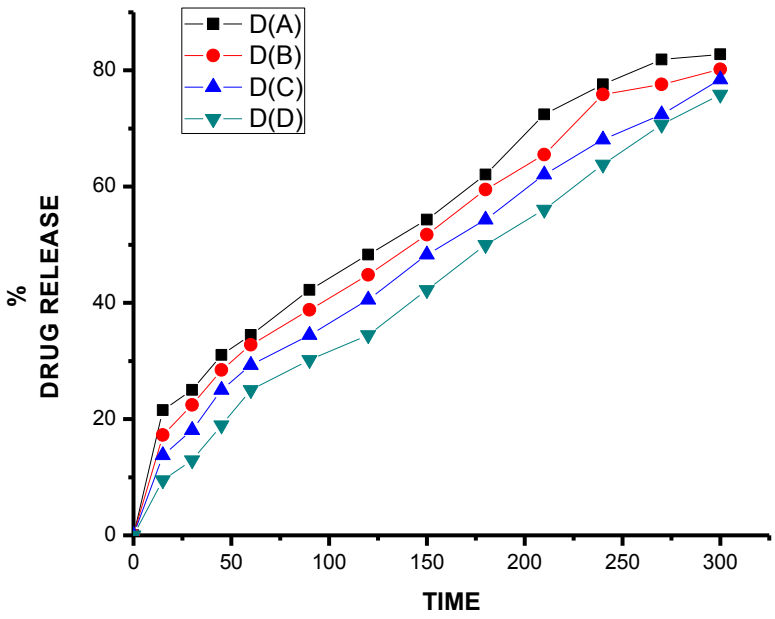


**Fig 10: Drug release study for various hydrogels**

# SUMMARY AND CONCLUSION

# Summary and Conclusion

Hydrogels of PEG and gelatin of four different volume ratio (gelatin: PEG) compositions were prepared. The compositions (gelatin: PEG) were 7:13, 8:12, 10:10 and 15:5 respectively. The stock solution prepared were 10% w/v each of PEG and gelatin. The hydrogels were characterized by FT-IR to determine the linkages and bonds in the hydrogels. The biocompatibility evaluation was done by swelling studies, mucoadhesive test, anti-microbial evaluation and haemocompatibility. The following conclusions were arrived from the research study:

- ✓ Swelling studies showed that samle A (high gelatin content) showed high swelling ratio when compared to other samples for the same time period.
- ✓ From mucoadhesive studies, it was found that sample D (less gelatin) disintegrated from the mucus layer at 20 h. All the other samples remained adhered to the mucus layer.
- ✓ Antimicrobial test showed that the drug loaded hydrogels exhibited more zone of inhibition when compared to blank (without drug) hydrogels.
- ✓ The percentage of haemolysis of all the four hydrogels was found to be less than 5 percent which indicates high haemocompatibility.
- ✓ Drug release was performed in dissolution test apparatus. It was found that drug release increase with respect to the increase of volume ratio of gelatin to PEG.

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